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Update on endodontic microbiology: candidate pathogens and patterns of colonisation

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The unequivocal role of microorganisms in the aetiology of apical periodontitis has been well established. As a consequence, successful treatment of this disease is contingent upon effective elimination or maximal reduction of the involved microbiota. There are at least two major aspects of endodontic infection that need to be well understood for establishing effective therapeutic protocols: the composition of the endodontic microbiota associated with the different forms of apical periodontitis and different types of infection (i.e. which are the main candidate pathogens); and the patterns of microbial colonisation within the root canal system (i.e. how they are distributed along the infected tissues). This review focuses on the current knowledge of these two aspects. Proper interpretation and clinical application of this information has the potential to be translated into improvements in quality and predictability of the treatment offered to patients.

Introduction

Endodontic infection is the infection of the root canal system and is the major aetiological agent of apical periodontitis. Although fungi, and most recently archaea and viruses, have been found in association with endodontic infection, bacteria are the major microorganisms implicated in the pathogenesis of apical periodontitis.

The endodontic infectious process usually takes place following pulp necrosis as a result of caries, trauma or iatrogenic procedures. The root canal containing a necrotic pulp then affords microorganisms a moist, warm, nutritious and anaerobic environment, which is by and large protected from the host defences. Such conditions are highly propitious for the establishment of a mixed microbiota conspicuously dominated by anaerobic bacterial species, most of which are usually normal inhabitants of the oral cavity. Therefore, endodontic infection is regarded as endogenous infection. In advanced stages of the infectious process, bacterial organisations resembling biofilms can be observed adhered to the canal walls. Thus, there is a current trend to include apical periodontitis in the category of biofilm-induced diseases (for a review on endodontic biofilms, see Ozok et al).
Bacteria colonising the root canal system enter in contact with the periradicular tissues via apical and lateral foramens. As a consequence of the encounter between bacteria and host defences, inflammatory changes take place in the periradicular tissues and give rise to the diverse forms of apical periodontitis. Although the original source of infection cannot be eliminated because of the absence of an active microcirculation in the necrotic pulp tissue, the host mounts a defence response adjacent to the apical foramen, in an attempt to prevent the infection from spreading into bone tissue and beyond. In some cases, depending on the intensity of tissue damage induced by microorganisms egressing from the root canal system, an acute apical abscess can form and the infection can even reach other anatomical sites in the head and neck. However, in most cases a balance between aggression and defence is obtained, which results in the development of a chronic inflammatory disease in the tissue around the portals of exit of microorganisms. As microorganisms infecting the root canal are inaccessible to both host defences and systemically administered antibiotics, apical periodontitis lesions are not self-healing and must be treated by means of professional intervention.

Given the infectious aetiology of apical periodontitis, successful treatment of this disease is contingent upon effective elimination of the endodontic microbiota. There are at least two major aspects of endodontic infection that need to be well understood for therapeutic purposes: the composition of the endodontic microbiota associated with the different forms of apical periodontitis and different types of infection; and the patterns of microbial colonisation within the root canal system. Knowledge of the microbial species involved in the aetiology of apical periodontitis aids in the selection of appropriate therapeutic measures, which should ideally involve substances that are effective against the major pathogens. Knowledge of the patterns of microbial colonisation in endodontic infection allows the establishment of antimicrobial therapeutic strategies to reach and eliminate microorganisms located not only in the main canal, but also in other areas of the system in which bacteria can propagate. If perfect therapies were available to treat apical periodontitis, with a success rate of 100% of the cases, there would be no practical need to understand these aspects of endodontic infection. As such therapies do not yet exist, endodontists will clearly benefit from this knowledge. In this context, a thorough understanding of the microbiological aspects related to apical periodontitis is crucial for modern, high-quality endodontics. This review focuses on these two important aspects of endodontic infection.

Microbial causation of apical periodontitis

Before further discussion of current aspects of endodontic infection, there follows a brief overview of the classic studies that helped to establish the foundation of contemporary endodontics as the discipline primarily involved with the treatment of an infectious disease.

The first recorded observation of bacteria in the root canal dates back to the 17th century, by the Dutch amateur microscope builder Antony van Leeuwenhoek (1632–1723). He reported that the root canals of a decayed tooth ‘were stuffed with a soft matter’ and that ‘the whole stuff’ seemed to him to be alive. However, the role of Leeuwenhoek’s ‘animalcules’ in disease causation was unsuspected at that time and it took almost 200 years before his observation was confirmed and a cause-and-effect relationship between bacteria and apical periodontitis was suggested.

In 1894, Willoughby Dayton Miller, an American dentist working at the laboratory of Robert Koch, in Berlin, Germany, published a milestone study reporting the association between bacteria and apical periodontitis after the analysis of samples collected from root canals. By means of bacterioscopy of the canal samples, he found bacterial cells in the three basic morphologies known, i.e. cocci (round cells), bacilli (cylindrical cells) and spirilla (helical cells). Morphologically, the endodontic microbiota was clearly different in the coronal, middle and apical parts of the root canal. Most of the bacteria he had seen under light microscopy could not be cultivated using the technology available at his time. Most of those bacteria were conceivably anaerobic bacteria, which were only successfully cultivated about 50 to 100
years later with the advent of anaerobic culture techniques. However, it is now widely recognised that a large number of bacterial species living in diverse environments still remain to be cultivated by current technology\textsuperscript{14,15}; the root canal is no exception (see below). Based on his findings, Miller raised the hypothesis that bacteria were the causative factors of apical periodontitis.

Only approximately 70 years after Miller’s classic study, his assumptions were confirmed by an elegant study from Kakehashi et al\textsuperscript{1}. These authors investigated the response of the dental pulps of conventional and germ-free rats after exposure to the oral cavities. Histological evaluation was performed, and revealed that whereas pulp necrosis and apical periodontitis lesions developed in all conventional rats, the pulps of germ-free rats not only remained vital but also repaired themselves by hard tissue formation. Dentine-like tissue sealed the exposure area and isolated the pulps again from the oral cavity.

The important role of bacteria in the aetiology of apical periodontitis was further confirmed by Sundqvist’s classic study\textsuperscript{2}. This author applied advanced anaerobic culturing techniques to the evaluation of bacteria occurring in the root canals of teeth whose pulps became necrotic after trauma. Bacteria were found only in the root canals of teeth exhibiting radiographic evidence of apical periodontitis, confirming the infectious aetiology of this disease. Anaerobic bacteria comprised more than 90\% of the isolates. Findings from Sundqvist’s study also served to demonstrate that, in the absence of infection, the necrotic pulp tissue itself and stagnant tissue fluid in the root canal cannot induce and perpetuate apical periodontitis lesions.

Möller et al\textsuperscript{16} also provided strong evidence for the microbial causation of apical periodontitis. Their study using monkey’s teeth demonstrated that only devitalised pulps that were infected induced apical periodontitis lesions, whereas devitalised and non-infected pulps showed an absence of significant pathological changes in the periapical tissues. In addition to corroborating the importance of microorganisms for the development of apical periodontitis, this study also confirmed that the necrotic pulp tissue per se is not able to induce and maintain an apical periodontitis lesion.

### Endodontic infection: definitions

Endodontic infection can be classified according to the anatomical location (intraradicular or extraradicular infection) and the time participating microorganisms established themselves in the root canal (primary, secondary, or persistent infection)\textsuperscript{17}.

The composition of the microbiota may vary depending on the different types of infection and different forms of apical periodontitis (Table 1). Delineation of the various types of endodontic infection favours the understanding of the pathological processes involving different clinical conditions and may help establish customised effective therapeutic measures for each condition.

Intraradicular infection is caused by microorganisms colonising the root canal system. It can be subdivided into three categories according to the time the microorganisms entered the root canal system:

- primary infection – caused by microorganisms that initially invade and colonise the necrotic pulp tissue (initial or ‘virgin’ infection)
- secondary infection – caused by microorganisms that were not present in the primary infection, but that were introduced in the root canal at some time after professional intervention (so called because it is secondary to intervention)
- persistent infection – caused by microorganisms that were members of a primary or secondary infection and that in some way resisted intracanal antimicrobial procedures, and were able to endure periods of nutrient deprivation in treated canals.

Both persistent and secondary infection are for the most part clinically indistinguishable, except for cases where signs and/or symptoms of infection arise in a previously non-infected tooth – a typical example of secondary infection. Extraradicular infection is characterised by microbial invasion of the inflamed periradicular tissues, occurring after the intraradicular infection. Extraradicular infection can be dependent on or independent of the intraradicular infection.
Composition of the endodontic microbiota

Modern anaerobic culture and sophisticated molecular biology techniques have disclosed important aspects of the endodontic microbiota in the different types of infection. Of particular interest, they have allowed the assessment of the microbial diversity in endodontic infection, collectively revealing that about 400 different microbial species can be found in infected root canals, usually in combinations involving many species in primary infection and a few species in secondary/persistent infection. Since endodontic infection develops in a previously sterile place, which as such does not contain a normal microbiota, any one of these species has the potential to be an endodontic pathogen or at least to play a role in the ecology of the endodontic microbial community. Culture and molecular studies reveal the prevalence of species, and consequently only association can be considered. Causation is usually surmised on the basis of both frequency of detection and potential pathogenicity (in animal models or inferred from association with other human diseases). Based on this, several species have emerged as candidate or putative endodontic pathogens. This section summarises the main current information on this topic.

Primary endodontic infection

It has been recently shown that primary infection is characterised by a mixed consortium composed of 10 to 30 species per canal. The bacterial load varies from $10^4$ to $10^8$ cells per canal. A positive relationship has been reported between the size of the apical periodontitis lesion and the number of cells and species in the infected canal. In other words, the larger the lesion the more complex the associated microbiota (Fig 1).

Although a large number of bacterial species (about 100 to 200) can be found in the oral cavity of a particular individual, only a limited set of these species (about 10 to 30) is consistently selected for growth and survival within a root canal containing necrotic pulp tissue from the same individual. This indicates that ecological determinants operate in the necrotic canal and dictate which species will succeed.
in colonising this previously sterile environment. The main ecological factors influencing the composition of the endodontic microbiota are oxygen tension, type and amount of available nutrients and bacterial interactions25.

Anaerobic bacteria dominate the mixed consortium. The most frequently detected culturable species in primary infection belong to the Gram-negative genera Tannerella, Dialister, Porphyromonas, Prevotella, Fusobacterium, Campylobacter and Treponema. Gram-positive anaerobes from the genera Peptostreptococcus, Parvimonas, Eubacterium, Filifactor, Actinomyces, Propionibacterium and Pseudoramibacter, as well as facultative or microaerophilic streptococci, can also be commonly found in primary infection19,26-68.

Bacterial profiles of the endodontic microbiota vary from individual to individual20, i.e. each individual harbours a unique endodontic microbiota in terms of species richness and abundance. This indicates that primary apical periodontitis has a heterogeneous aetiology, where no single species can be considered as the main endodontic pathogen, and multiple bacterial combinations play a role in disease causation.

Culture-independent molecular biology studies have demonstrated that as-yet-uncultivated bacteria can participate in endodontic infection – more than 40–55% of the endodontic microbiota is composed of bacterial phylotypes, i.e. species that are known only by a 16S rRNA gene sequence and that have yet to be cultivated and fully characterised19,44. Several uncultivated phylotypes from the genera Synergistes, Dialister, Megasphaera, Solobacterium, Eubacterium and Selenomonas, as well as phylotypes related to the family Lachnospiraceae or the phylum Bacteroidetes have been identified in primary endodontic infection19,44,45,47,50,69,70. The fact that these bacterial phylotypes are uncultivated only means that we cannot grow them artificially in the laboratory with the culture media and techniques available. They are certainly flourishing in their natural environment, where essential nutrients, growth factors and other favourable conditions are fully available. Therefore, there is no reason to believe that they are less important than the cultivable proportion of the microbiota when it comes to disease causation.

Acute apical periodontitis and acute apical abscesses are typical examples of symptomatic endodontic infection. In these cases, the infection is located in the root canal, but it has also reached the periradicular tissues and, in abscessed cases, it can spread to other anatomical spaces. Whereas microbial causation of apical periodontitis is well established, there is no strong evidence disclosing specific involvement of a single species with any particular sign or symptom of apical periodontitis. Some Gram-negative anaerobic bacteria have been suggested to be involved with symptomatic lesions2,33,44,65,71-73, but the same species may also be present in somewhat similar frequencies in asymptomatic cases28,30,34,37,40,74. Therefore, factors other than the mere presence of a given putative pathogenic species may play a role in the aetiology of symptomatic endodontic infection75,76. These factors include: differences in virulence ability among strains of the same species; bacterial interactions resulting in synergism or additive effects among species in mixed infection; number of bacterial cells (load); environmental cues regulating expression of virulence factors; host resistance; and concomitant herpesvirus infection. Association of some or all of these factors (instead of an isolated event) is likely to determine the occurrence and intensity of symptoms75,76.
Because apical periodontitis is a disease of infectious aetiology, the logical goals of the endodontic treatment are to eliminate or substantially reduce the microbial populations within the root canal system (through antiseptic means) and to prevent introduction of new microorganisms in the canal (through aseptic means). Prevention of reinfection is also achieved by a tight coronal seal of the root canal provided by both the root canal filling and the permanent coronal restoration. The success rate of the endodontic treatment will depend on how effective the clinician is in accomplishing these goals.

Persisting or secondary intraradicular infection is the major cause of several clinical problems, such as persistent exudation, persistent symptoms, flare-ups and treatment failure. The latter is characterised by persistence or appearance of apical periodontitis after treatment (Fig 2). Microorganisms present in root-canal-treated teeth can be ‘persisters’ that survived the effects of intracanal disinfection procedures and were present in the canal at the root-canal-filling stage (persistent intraradicular infection), or they can have infected the canal after filling as a result of coronal leakage (secondary intraradicular infection). The high success rate of the treatment of vital (non-infected) teeth strongly suggests that persistent infection can be the most common cause of failure in the treatment of teeth with apical periodontitis. Should a secondary infection caused by coronal leakage be the most significant cause of post-treatment disease, the failure rates for the treatment of vital or necrotic teeth or even retreatment cases would be similar – but they differ greatly

The concept of coronal leakage as an important cause of failure is further put into question by the findings of a study that revealed that well-prepared and sealed canals resisted coronal bacterial leakage even upon complete oral exposure for prolonged periods. However, this does not mean that the attainment of a good coronal seal is not a goal of the endodontic treatment, since coronal leakage in obtu-
rated canals can still be the cause of failure in some cases, and the clearest example seems to be those cases where an apical periodontitis lesion was absent at the time of treatment but which appeared on follow-up radiographs.

Microbial involvement with treatment failures is supported by two strong evidence-based arguments. First, it has been demonstrated that there is an increased risk of adverse treatment outcome when bacteria are present in the canal at the time of filling\textsuperscript{80-82}. This reinforces the concept that persistent intraradicular infection can be the major cause of failure as compared to secondary infection (coronal leakage). Secondly, most (if not all) root-canal-treated teeth with persistent apical periodontitis lesions have been demonstrated to harbour an intraradicular infection\textsuperscript{83-88}. Based on these arguments, studies have attempted to identify the microorganisms found at the root-canal-filling stage, which may jeopardise the treatment outcome (outcome into perspective), and the microorganisms in root-canal-treated teeth with apical periodontitis, which are arguably the cause of failure (outcome already established).

**Bacteria at the root-canal-filling stage**

Root canal samples positive for bacterial growth after chemo-mechanical procedures followed or not by intracanal medication have been shown to harbour an average of one to five bacterial species per case, with counts reaching $10^2$ to $10^5$ cells per sample\textsuperscript{21,23,81,89-91}. This indicates that, even if total bacterial elimination is not the case, at least a substantial reduction in species richness and abundance is attained. No single species has been significantly found to persist after treatment procedures. Gram-negative bacteria, which are common members of primary infection, are usually eliminated. Most studies have clearly revealed that, when bacteria resist treatment procedures, Gram-positive bacteria are more frequently present. They include streptococci, *Parvimonas micra* (previously *Peptostreptococcus/Micromonas micros*), *Actinomyces* species, *Propionibacterium* species, *Pseudoribabacter* *lactolyticus*, lactobacilli, *Enterococcus faecalis* and *Olsenella ulii*\textsuperscript{21,81,89-97}. About 40\% of the taxa found in post-treatment samples are as-yet-uncultivated bacteria\textsuperscript{21}, indicating that, along with other reasons (such as inaccessibility to sampling or low levels), attainment of negative cultures does not necessarily imply sterility.

**Microbiota in root-canal-treated teeth**

The microbiota in root-canal-treated teeth with persistent apical periodontitis lesions also exhibits a decreased diversity in comparison to primary infection. Canals apparently well treated contain one to five species, while the number of species in canals with inadequate treatment can reach up to 30 species, which is very similar to untreated canals\textsuperscript{86-88,98}. An individual treated canal associated with post-treatment disease can harbour a density of $10^2$ to $10^7$ bacterial cells\textsuperscript{93,99}.

Culture-dependent and culture-independent methods have revealed that *E. faecalis* is the species most frequently found in root-canal-treated teeth, with prevalence values reaching up to 90\% of the cases\textsuperscript{95-88,99-102}. Root-canal-treated teeth are about nine times more likely to harbour *E. faecalis* than cases of primary infection\textsuperscript{102}. This suggests that this species can be inhibited by other members of a mixed bacterial consortium commonly present in primary infection, and that the bleak environmental conditions within filled root canals do not prevent its survival.

For a given microorganism to survive in treated canals, it has to resist intracanal disinfection procedures and to adapt to the harsh environmental conditions caused by treatment. *E. faecalis* has the ability to penetrate dentinal tubules, sometimes to a deep extent\textsuperscript{103,104}, and this property can enable it to escape from the action of endodontic instruments and irrigants used during chemo-mechanical preparation\textsuperscript{103,105}. Moreover, the ability of *E. faecalis* to form biofilms in root canals can be important for its resistance to and persistence after intracanal antimicrobial procedures\textsuperscript{106}. *E. faecalis* is also resistant to calcium hydroxide\textsuperscript{107}, a commonly used interappointment medicament. Unlike most putative endodontic pathogens that are frequently found in primary infection, *E. faecalis* may colonise root canals as a single infection\textsuperscript{87}, and such a relative independence of living without deriving nutrients from other bacteria can be extremely important for its establishment in treated root canals. Finally, environmental
cues can regulate gene expression in *E. faecalis*, affording this bacterium the ability to adapt to varying (and adverse) conditions\textsuperscript{108}. Indeed, *E. faecalis* can enter a viable but non-culturable (VBNC) state\textsuperscript{109}, which is a survival mechanism adopted by several bacteria when exposed to unfavourable environmental conditions\textsuperscript{110}. In the VBNC state, bacteria lose the ability to grow in culture media but maintain viability and pathogenicity, and sometimes can resume division when optimal environmental conditions are restored. *E. faecalis* has the ability to survive in environments with a scarcity of nutrients and to flourish when the nutrient source is reestablished\textsuperscript{111}. For instance, it was demonstrated that *E. faecalis* has the capacity to recover from a prolonged starvation state in obturated canals\textsuperscript{112}, suggesting that viable cells of this species entombed at the time of root filling may provide a long-term nidus for subsequent infection.

Taken together, all these properties help explain the significantly high prevalence of *E. faecalis* in root-canal-treated teeth. While association of this species with post-treatment disease is suggested by epidemiological studies and supported by the species attributes that allow it to survive under unfavourable environmental conditions, causation is not proved. In fact, the status of *E. faecalis* as the main causative agent of endodontic failures has been recently questioned by the following findings from studies carried out in independent laboratories:

- in spite of being easily cultured, *E. faecalis* is not detected in all studies evaluating the microbiota of root-canal-treated teeth with lesions\textsuperscript{70,113}
- even when present, *E. faecalis* is rarely one of the most dominant species in retreatment cases\textsuperscript{98}
- *E. faecalis* has been found not to be more prevalent in root-canal-treated teeth with lesions when compared to teeth with no lesions\textsuperscript{101,114}.

Other bacteria found in root-canal-treated teeth with apical periodontitis include streptococci and some fastidious anaerobic bacterial species – *P. acal- tolyticus, Propionibacterium propionicum, Filifac- tor alocis, Dialister pneumosintes and Dialister invi- sus*\textsuperscript{47,86-88,98,100}. As-yet-uncultivated phylotypes correspond to 55% of the taxa detected in treated canals\textsuperscript{115}. The bacterial community profiles in treated cases vary from individual to individual, suggesting that distinct bacterial combinations can play a role in treatment failures\textsuperscript{98}. All these findings strongly suggest that the microbiota of root-canal-treated teeth with apical periodontitis is more complex than previously anticipated by culture studies.

Fungi are only occasionally found in primary infection, but *Candida* species have been detected in root-canal-treated teeth in up to 18% of the cases\textsuperscript{96-98,99,100,113,116,117}. Fungi gain access to root canals via contamination during endodontic therapy or they overgrow after inefficient intracanal antimicrobial procedures that cause an imbalance in the primary endodontic microbiota\textsuperscript{5}. *Candida albicans* is by far the most commonly detected fungal species in root-canal-treated teeth. This species has several properties that can be involved in persistence following treatment, including ability to colonise and invade dentine\textsuperscript{118-120} and resistance to calcium hydrox-ide\textsuperscript{121,122}.

### Extraradicular infection

Apical periodontitis lesions are formed in response to intraradicular infection and for the most part comprise an effective barrier against spread of the infection to the alveolar bone and other body sites. Nevertheless, in some specific circumstances, microorganisms can overcome this defence barrier and establish an extraradicular infection, which can be dependent on or independent of the intraradicular infection\textsuperscript{123}.

The question as to whether the extraradicular infection is dependent on or independent of the intraradicular infection has special relevance from a therapeutic standpoint. For instance, the acute apical abscess is usually dependent on the intraradicular infection: once the intraradicular infection is properly controlled by root canal treatment or tooth extraction and drainage of pus is achieved, the extraradicular infection is handled by the host defences and usually subsides. The extraradicular occurrence of several anaerobic bacteria has also been reported in recalcitrant chronic apical periodontitis lesions\textsuperscript{124-126}, but there is no evidence that these bacteria were actually established as an extraradicular infection independent of the intraradicular infection.

Apical actinomycosis, in turn, has been claimed to be independent of the intraradicular infection in the
sense that even if the treatment succeeds in eradicating intraradicular microorganisms, the lesion cannot heal because the causative agents are already beyond the reaches of non-surgical root canal treatment procedures. Thus, apical actinomycosis, which is caused by *Actinomyces* species or *P. propionicum*, is successfully treated only by periradicular surgery, and use of systemic antibiotic therapy is for the most part not necessary.\(^{123,127,128}\)

The incidence of independent extraradicular infection in untreated teeth is conceivably low, which is congruent with the high success rate of non-surgical root canal treatment.\(^7,77\) For instance, apical actinomycosis accounts for only 1.8–4% of the apical periodontitis lesions.\(^{123}\) Even in root-filled teeth with recalcitrant lesions, in which a higher incidence of extraradicular bacteria has been reported,\(^{124,125}\) the high rate of healing following retreatment indicates that the major cause of post-treatment disease is located within the root canal system, characterising a persistent or secondary intraradicular infection.

**Herpesviruses in extraradicular infection**

Viruses require viable host cells to infect and replicate themselves; hence they cannot survive in the root canal with necrotic pulp. Viruses have been reported to occur in the canal only in non-inflamed vital pulps of patients infected with the human immunodeficiency virus (HIV).\(^{129}\) On the other hand, human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) have been detected in apical periodontitis lesions,\(^{6,7,10,130-132}\) where living host cells abound. Hypothetically, HCMV and EBV may be implicated in the pathogenesis of apical periodontitis as a direct result of viral infection and replication or as a result of virally induced impairment of local host defences, which might give rise to overgrowth of pathogenic bacteria in the very apical part of the root canal.\(^6\) Frequency of HCMV and EBV detection has been found to be high in the presence of symptoms,\(^{132,133}\) in lesions exhibiting elevated occurrence of anaerobic bacteria,\(^{130}\) in cases of large periradicular bone destruction,\(^{130,133}\) and in HIV-positive patients.\(^6\) The specific role played by herpesviruses in the pathogenesis of apical periodontitis still needs to be clarified.

**Patterns of microbial colonisation**

The knowledge of microbial location and organisation within the root canal system is especially important for the understanding of the disease process and the establishment of effective antimicrobial therapeutic strategies. Most of the knowledge of the structure of the endodontic microbiota comes from morphological studies,\(^8-10,134\) which do not, however, usually provide information as to the bacterial identity and numbers. Consequently, it is not possible to delineate the role of the visualised bacteria in the disease process. Thus, as each bacterial cell observed in the root canal system might be an endodontic pathogen, findings from morphological studies should be used to understand the topography of the root canal infection and to establish therapeutic measures in an attempt to completely eradicate the root canal infection or at least to reduce the bacterial load to thresholds that are compatible with peri-radicular tissue healing.

The root canal microbiota in primary infection is dominated by bacterial morphotypes, including cocci, rods, filaments and spirilla. Fungal cells are sometimes found.\(^5,134\) Most of the endodontic microbiota occurs in the main root canal (Figs 3 and 4), usually in planktonic state, i.e. suspended in a fluid phase. Dense bacterial aggregates can be seen attached to the root canal walls, sometimes forming...
multilayered bacterial biofilms\(^8,9\) (Fig 5). Lateral canals and isthmuses connecting main canals can also be clogged with microbial cells, primarily organised in biofilms\(^{135}\).

Bacteria forming a biofilm on the root canal walls are often seen penetrating the dentinal tubules (Fig 4). The diameter of dentinal tubules is large enough to permit penetration of most oral bacteria. It has been reported that dentinal tubule infection can occur in about 70–80% of teeth with apical periodontitis lesions\(^{136,137}\). Although a shallow penetration is more common, bacterial cells can be observed reaching approximately 300 μm in some teeth\(^9\). Dividing cells can be frequently observed within tubules in \textit{in situ} investigations\(^9\), indicating that bacteria can derive nutrients within tubules, probably from degrading odontoblastic processes, denatured collagen, bacterial cells that die during the course of infection and intracanal fluids that enter the tubules by capillarity.

Several putative endodontic pathogens have been shown to be capable of penetrating dentinal tubules \textit{in vitro}, including \textit{Porphyromonas endodontalis}, \textit{Porphyromonas gingivalis}, \textit{Fusobacterium nucleatum}, \textit{Actinomyces israelii}, \textit{Propionibacterium acnes}, \textit{E. faecalis}, \textit{C. albicans} and \textit{Streptococcus}\(^{104,120,138-140}\). In a clinical study, Peters et al\(^{137}\) isolated and identified bacteria present in root dentine at different depths, and the most common isolates belonged to the genera \textit{Prevotella}, \textit{Porphyromonas}, \textit{Fusobacterium}, \textit{Veillonella}, \textit{Peptostreptococcus}, \textit{Eubacterium}, \textit{Actinomyces}, \textit{Lactobacilli} and \textit{Streptococci}. Matsuo et al\(^{136}\), using immunohistological analysis, observed the intratubular occurrence of \textit{F. nucleatum}, \textit{P. alactolyticus}, \textit{Eubacterium nodatum}, \textit{Lactobacillus casei} and \textit{P. micra} inside dentinal tubules from the canal walls of extracted infected teeth with apical periodontitis.

While bacteria present as planktonic cells in the main root canal may be easily accessed and eliminated by instruments and substances used during treatment, those organised in biofilms attached to the canal walls or located into isthmuses, lateral canals and dentinal tubules are definitely more difficult to reach and may require special therapeutic strategies to be eradicated.
Concluding remarks

The unequivocal role of microorganisms in the aetiology of apical periodontitis was established almost 40 years ago. Recent years have witnessed a huge amount of new information about the main species involved with the different types of endodontic infection (Table 1), and the way microorganisms colonise the root canal system. This knowledge has the potential to be incorporated into clinical practice and be translated into improvements in the quality of treatment offered to our patients.

References


